Volume 15 Number 4 1987 Nucleic Acids Research

Adjustment of the tRNA population to the codon usage in chloroplasts

Hélène Pfitzinger, Pierre Guillemaut, Jacques-Henry Weil and D.T.N.Pillay[1]

Institut de Biologie Moléculaire et Cellulaire, Université Louis Pasteur, 15 Rue Descartes, 67084 Strasbourg, France, and [1]Department of Biology, University of Windsor, Windsor, Ontario N9B 3P4, Canada

Received December 30, 1986; Accepted January 27, 1987

ABSTRACT

In chloroplasts there is a correlation between the amounts of tRNAs specific for a given amino acid and the codons specifying this amino acid. Furthermore, for the amino acids coded for by more than one codon, the population of isoaccepting tRNAs is adjusted to the frequency of synonymous codons used in chloroplast protein genes. A comparison by two-dimensional gel electrophoresis of the tRNA populations extracted from chloroplasts and from chloroplast polysomes shows that all chloroplast tRNAs are involved in protein biosynthesis.

INTRODUCTION

In the universal genetic code, all amino acids except methionine and tryptophan are coded for by several (two to six) codons called synonymous codons. Although they are equivalent in terms of protein structure, synonymous codons are not used with equal frequency in DNA coding sequences. In **E.coli** and the yeast **S.cerevisiae** it has been shown that differences in the usage of synonymous codons are related to differences in the population of isoaccepting tRNAs. There is a strong positive correlation between codon usage and tRNA content in these two unicellular organisms. Furthermore highly expressed genes contain codons which correspond to major tRNA species, while genes that are expressed at low levels contain codons which correspond to minor tRNA species (for reviews see 1-3).

Plant cells contain mitochondria (which are found in all eukaryotic organisms) and chloroplasts : both are semi-autonomous organelles, able to synthesize specific proteins. Several observations have shown that translation processes in chloroplasts and bacteria are closely related, suggesting a prokaryotic origin for these organelles (for a review see 4). Recently it has been shown that only 30 different tRNA species are encoded by the higher plant (5,6) chloroplast genome, but 32 tRNA genes have been found in **Marchantia** (7). As all possible codons are found in the chloroplast DNA sequences coding for proteins, the minimum number of tRNA species required

for the translation of all codons is 32, according to the wobble hypothesis. Furthermore, as two tRNAs, namely $tRNA^{Leu}_{UAA}$ and $tRNA^{Leu}_{CAA}$ are able to decode the same codon UUG, there would be a deficit of 3 tRNAs to decode the sense codewords in higher plant chloroplasts. As no cytoplasmic tRNA seems to be imported into the chloroplast, a unique mechanism must operate in the chloroplasts to decode all 61 codons (4-6).

Previous studies in our laboratory have shown that in bean chloroplasts the concentrations of tRNAs specific for different amino acids are quite different (8) and that there are also differences in the concentrations of isoacceptors for a given amino acid (8-12). Similar results have been found for cotton (13).

In order to see whether there is a correlation between codon usage and tRNA content, we have compared the codon frequency in chloroplast protein genes with the abundance of the corresponding tRNAs in chloroplasts. Furthermore, in order to determine if all chloroplast tRNAs are actually used in protein synthesis, we have isolated the chloroplast polysome-bound tRNAs, fractionated the isoacceptors by two-dimensional polyacrylamide gel electrophoresis and compared the patterns obtained to that obtained with total chloroplast tRNAs.

MATERIALS AND METHODS

Chloroplasts were purified from bean leaves (**Phaseolus vulgaris**) as already described by Herrmann **et al.** (14). Chloroplast polysomes were isolated (15) from highly purified chloroplasts in a Percoll gradient (16) except that a transition-state analog complex of guanosine and vanadyl sulfate, which inhibits ribonuclease activity, was added to the medium (17). Total chloroplast tRNAs and polysome-bound tRNAs were obtained respectively from purified chloroplasts and from polysome pellets, using the method previously described (8). The tRNAs were labeled enzymatically at the 3' end using α-$|^{32}P|$ATP and yeast tRNA nucleotidyl transferase (18). Most of the individual tRNAs were obtained as pure species using two-dimensional polyacrylamide gel electrophoresis (2-D-PAGE) (19,20).

Aminoacylation of tRNAs was performed with chloroplast or **E.coli** enzymes using conditions which give complete charging of tRNAs (plateau value) (8-12). Isoacceptors, which had been previously fractionated in our laboratory (8-12) by RPC-5 chromatography (21) were identified upon co-chromatography of total chloroplast tRNAs aminoacylated with a given ^{14}C-amino acid and a pure isoacceptor (corresponding to the amino acid studied) aminoacylated with the

^{3}H-amino acid. The chromatographic conditions used were as previously described (8-12). On the elution pattern, the area corresponding to each aminoacylated tRNA peak was measured, allowing us to determine the percentage of each isoaccepting species for a given amino acid, either in total or polysome-bound tRNAs.

RESULTS AND DISCUSSION

1) Correlation between codon usage and tRNA content in chloroplasts

In order to see whether there is a correlation between codon usage and tRNA abundance in chloroplasts, we have compared on one hand the codon usage in chloroplast protein genes, recently reviewed in maize, spinach (4) and tobacco (5), and on the other hand the tRNA content in the chloroplasts of bean (extensively studied in our laboratory) and cotton (studied by Merrick and Dure). The amounts of bean chloroplast tRNAs which can be charged with each of the 20 amino acids have been measured by aminoacylation of total chloroplast tRNAs under conditions described in Materials and Methods. In the case of cotton chloroplast tRNAs, results previously published by Merrick and Dure (13) have been used after having taken into account the contamination of chloroplast tRNAs by cytoplasmic tRNAs. These authors estimated that cotton chloroplast tRNAs were contaminated by 20-25% cytoplasmic tRNAs. A more precise measure of these contaminations was obtained in several cases, where these authors have characterized, by chromatographic analysis, the cytoplasmic tRNA species present in their chloroplast tRNA preparations (13).

The results obtained are summarized in Table 1. These results clearly show that in chloroplasts there is a strong positive correlation between the relative abundance of the codons for each amino acid in chloroplast protein genes and the relative abundance of the corresponding tRNAs. For example, codons for leucine are the most abundant, while codons for tryptophan are the least abundant in chloroplast protein genes in tobacco, spinach and maize ; and, in both plants studied, the amounts of $tRNA^{Leu}$ are the highest, while the amounts of $tRNA^{Trp}$ are the lowest. In the case of the $tRNAs^{Met}$, there is a good correlation between the levels of $tRNA_{m}^{Met}$ and the abundance of the methionine elongation codon, whereas the levels of $tRNA_{f}^{Met}$ are much higher than expected from the abundance of the initiation codon ; this suggests that formyl-methionyl-$tRNA^{Met}$, which initiates protein biosynthesis in chloroplasts (22), has to be abundant in order not to be a limiting factor in the initiation of translation. But in general, it appears that, in chloroplasts, the relative amounts of the tRNAs specific for a given amino

Table 1. Codon Usage and tRNA Content in Chloroplasts.

tRNA corresponding to :	Relative abundance of codons corresponding to each amino acid on chloroplast protein genes (a,b)				Relative abundance of the tRNAs in chloroplasts (a,c)	
	Tobacco (d)	Maize (e)	Spinach (f)	*Average*	Bean (g)	Cotton (h)
Ala	0,76	0,79	0,82	0,79	-	0,57
Arg	0,67	0,46	0,46	0,53	0,62	0,87
Asn	0,41	0,32	0,31	0,35	0,59	0,26
Asp	0,37	0,43	0,37	0,39	0,20	0,62
Cys	0,08	0,067	0,096	0,081	-	-
Gln	0,37	0,35	0,26	0,33	-	-
Glu	0,52	0,39	0,46	0,46	0,60	0,37
Gly	0,87	0,87	1	0,92	0,85	1
His	0,26	0,37	0,22	0,29	0,32	0,44
Ile	0,79	0,65	0,53	0,65	0,79	0,58
Leu	1	1	1	1	1	1
Lys	0,47	0,37	0,32	0,39	0,48	0,51
Met_f (i)	0,027	0,023	0,022	0,023	0,21	0,26
Met_m (i)	0,23	0,21	0,20	0,21	0,26	0,39
Phe	0,48	0,51	0,66	0,55	0,45	0,55
Pro	0,45	0,40	0,54	0,46	0,42	0,40
Ser	0,62	0,57	0,50	0,57	0,59	0,50
Thr	0,56	0,58	0,54	0,56	0,39	0,57
Trp	0,18	0,24	0,24	0,22	0,25	0,22
Tyr	0,32	0,29	0,31	0,31	0,36	0,37
Val	0,64	0,51	0,77	0,64	0,70	0,62

a) Codons and tRNAs found in the highest amounts have been normalized at 1.0
b) For each amino acid, all codons were taken into account. In the case of Met, we considered that out of ten AUG codons, one was an initiation codon and nine an elongation codon (4).
c) The amounts of the various tRNAs were estimated by aminoacylation of total chloroplast tRNAs with each amino acid at the plateau value.
d) 10,217 codons in 39 protein genes were considered (5).
e) 2675 codons in 5 protein genes were considered (4).
f) 2533 codons in 8 protein genes were considered (4).
g) Our results.
h) Results previously published by Merrick and Dure (13).
i) The amounts of $tRNA_f^{Met}$ and $tRNA_m^{Met}$ have been determined by measuring the relative area of each isoacceptor peak upon fractionation and identification by chromatographic analysis (9,13).

acid are closely related to the abundance of the codons specifying this amino acid.

2) Synonymous codon usage and relative content of isoaccepting tRNAs in bean chloroplasts

As all amino acids except methionine and tryptophan are coded for by several synonymous codons, we have examined for several amino acids whether a correlation exists between the frequency of codon usage in chloroplast protein genes and the amounts of the corresponding isoacceptors (identified and quantified as described in Materials and Methods).

The results obtained are summarized in Table 2. It appears that for the four amino acids studied, which are coded for by more than one codon, the tRNA pool is adapted to the frequency of codon usage in the corresponding family. There is a very good correlation between the usage of synonymous codons and the relative concentrations of the corresponding isoaccepting tRNAs in the chloroplasts.

In the case of the leucine codons, it is not known whether the UUG codon is read by $tRNA^{Leu}_{UAA}$ and/or by $tRNA^{Leu}_{CAA}$. It has been suggested that $tRNA^{Leu}_{CAA}$ could be dispensable, since $tRNA^{Leu}_{UAA}$ could also read the codon UUG (4,5). However we have found both $tRNA^{Leu}_{UAA}$ and $tRNA^{Leu}_{CAA}$ on polysomes isolated from chloroplasts (see below), which strongly suggests that $tRNA^{Leu}_{CAA}$ is required to read UUG codons in chloroplast mRNAs. Furthermore the relative content of $tRNA^{Leu}_{CAA}$ exactly fits the UUG codon usage (22% and 21% respectively), whereas the relative content of $tRNA^{Leu}_{UAA}$ fits the UUA codon usage (36% in both cases), suggesting that it reads only UUA and not UUG. Our hypothesis is that the unknown modified nucleotide found in the wobble position of $tRNA^{Leu}_{UAA}$ (24) restricts the reading of this tRNA to UUA. This hypothesis is in agreement with earlier data indicating that some modifications of U in the wobble position of a tRNA can restrict the recognition properties of this tRNA to codons ending with A (1,2,25).

3) Studies on bean chloroplast tRNAs involved in protein biosynthesis

In order to study the utilization of bean chloroplast tRNAs in protein biosynthesis, we have isolated chloroplast polysomes and compared polysome-bound tRNAs to total chloroplast tRNAs. These analyses have been done by 2-D-PAGE and by RPC-5 chromatography, as described under Materials and Methods. As shown in figure 1, the patterns obtained by 2-D-PAGE analysis of total (fig. 1A) and polysome-bound (fig. 1B) bean chloroplast tRNAs labeled with $|^{32}P|$ are very similar (they show 26 and 25 radioactive spots respectively). All these radioactive spots correspond to methylene blue-stained tRNA spots,

Table 2. Synonymous codon usage and relative content of isoaccepting species in chloroplasts

tRNA corresponding to :	Anticodons (b)	Codons	Codons usage in chloroplasts % (a)	Relative amounts of each isoacceptor in its tRNA family (in bean chloroplasts) %
Arg (c)	UCU	AGA AGG	31	26
Arg (g)	ACG	CGU CGC CGA CGG	69	74
Gly (d)	GCC	GGU GGC	49	50
Gly	UCC	GGA GGG	51	50
Ile	CAU (e)	AUA	26	32
Ile	GAU	AUU AUC	74	68
Leu (f)	UAA	UUA(UUG)	36	36
Leu	CAA	UUG	21	22
Leu (g)	UAG	CUU CUC CUA CUG	43	42

a) Codon usage in the chloroplasts of tobacco, maize and spinach was taken into account, as in table 1, but here the values represent the percentage of each codon in the family of synonymous codons.
b) Possible nucleotide modifications have not been considered
c) Fractionation and identification of bean chloroplast $tRNAs^{Arg}$ have been done recently in our laboratory (data not shown).
d) Separation of bean chloroplast $tRNAs^{Gly}$ has been done by Sepharose-4B chromatography by J. Canaday (unpublished results).
e) Modification of C allows recognition of the isoleucine codon AUA and not of the methionine codon AUG (23).
f) Calculations have be done assuming that $tRNA^{Leu}_{UAA}$ only reads the codon UUA.
g) Codon reading is according to ref. 4-6.

except spot 25 on the total tRNA gel (fig. 1A). This spot has been shown to contain a series of oligonucleotides which probably derive from damaged tRNAs. Most of the tRNAs found in the radioactive spots have been identified (data not shown) by aminoacylation and/or sequence determination of their 3'-end by homochromatography (18). The pattern obtained is in good agreement with that previously published (20). The number of distinct spots obtained (25 spots) is lower than the number of tRNAs (30) expected from the gene sequence data (5,6). But is has been shown previously (19) that one spot can contain several tRNAs which migrate together in the two dimensions. It also should be pointed out that $tRNA^{Asn}$, which in tobacco is coded for by one gene, has been found in three spots, namely spots 14, 19 and 19'. Previous studies had already shown the presence of $tRNA^{Asn}$ in two spots (20). The

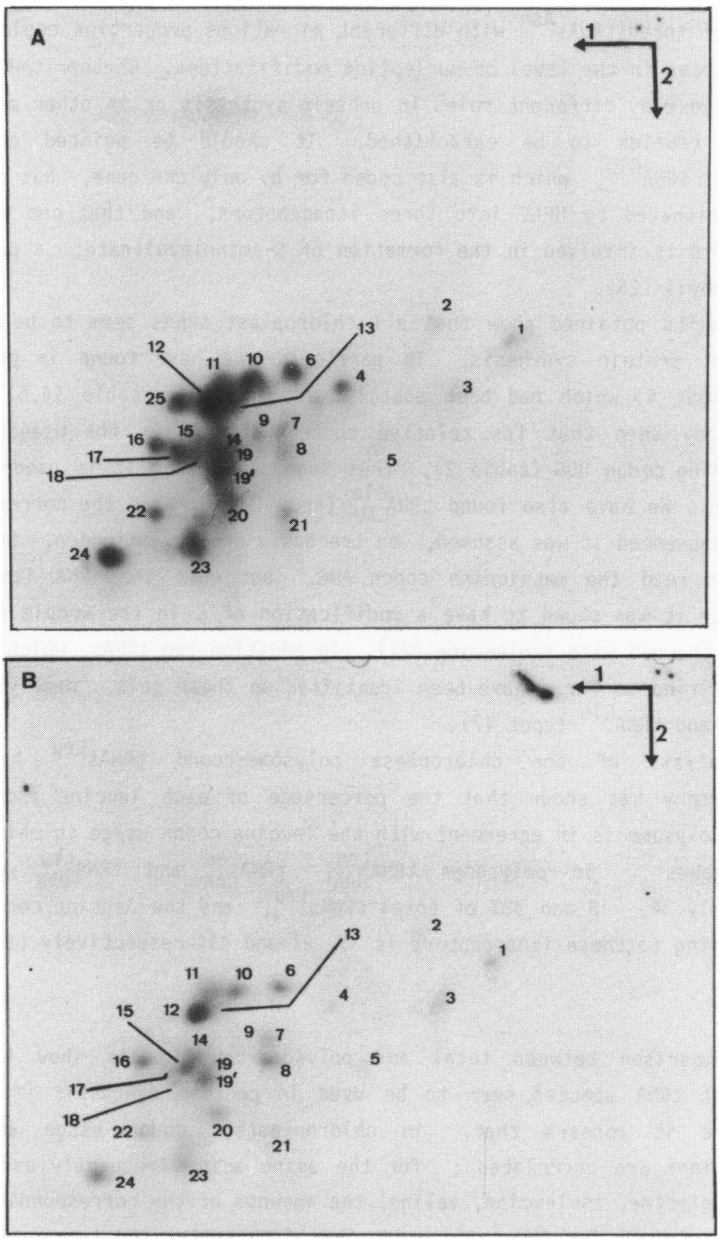

Fig. 1. Autoradiograms of 2-D-PAGE fractionation of bean chloroplast total tRNAs (A) and polysome-bound tRNAs (B) labeled with $|^{32}P|$.
0.5 µg of total (gel A) or polysome-bound (gel B) tRNAs labeled with $|^{32}P|$ was added to 150 µg of unlabeled total chloroplast tRNAs. The tRNA mixture was then fractionated by 2-D-PAGE as described in "Materials and Methods". After electrophoresis, the gel was stained with methylene blue and autoradiographed for 15 hours.

presence of three $tRNAs^{Asn}$ with different migrations properties could be due to differences in the level of nucleotide modifications. Whether these three $tRNAs^{Asn}$ can play different roles in protein synthesis or in other metabolic processes remains to be established. It should be pointed out that chloroplast $tRNA^{Glu}$, which is also coded for by only one gene, has recently been fractionated by HPLC into three isoacceptors, and that one of these isoacceptors is involved in the formation of S-aminolevulinate, a precursor of chlorophyll (26).

The results obtained show that all chloroplast tRNAs seem to be used in chloroplast protein synthesis. In particular we have found in polysomes $tRNA^{Leu}_{CAA}$ (spot 4) which had been postulated to be dispensable (4,5) but we have already seen that its relative concentration fits the usage of the corresponding codon UUG (table 2), thus suggesting that it is used to read this codon. We have also found $tRNA^{Ile}_{CAU}$ (spot 23) ; when the corresponding gene was sequenced it was assumed, on the basis of its anticodon, that this tRNA would read the methionine codon AUG, but when the tRNA itself was sequenced, it was shown to have a modification of C in the wobble position and to be charged with isoleucine (21). In addition two tRNAs, which had not been identified so far, have been identified on these gels, namely $tRNA^{Cys}$ (spot 11) and $tRNA^{Gln}$ (spot 17).

An analysis of the chloroplast polysome-bound $tRNAs^{Leu}$ by RPC-5 chromatography has shown that the percentage of each leucine isoacceptor bound to polysome is in agreement with the leucine codon usage in chloroplast protein genes : In polysomes $tRNA^{Leu}_{UAA}$, $tRNA^{Leu}_{CAA}$ and $tRNA^{Leu}_{UAG}$ represent respectively 34, 18 and 48% of total $tRNAs^{Leu}$, and the leucine codon usage corresponding to these isoacceptors is 36, 21 and 43% respectively (table 2).

CONCLUSION

The comparison between total and polysome-bound tRNAs show that all chloroplast tRNA species seem to be used in protein synthesis (fig. 1). Furthermore it appears that, in chloroplasts, codon usage and tRNA concentrations are correlated ; for the amino acids frequently used (e.g. leucine, glycine, isoleucine, valine) the amounts of the corresponding tRNAs are high, whereas for the amino acids less frequently used (e.g. tyrosine, histidine, tryptophan) the corresponding tRNA concentrations are low (table 1). On the other hand for each amino acid specified by more than one codon, the isoaccepting tRNA pool is adapted to the codon usage in the family of synonymous codons (table 2).

It has been shown that, in chloroplasts, several tRNA genes contain long introns, namely $tRNA^{Leu}_{UAA}$, $tRNA^{Ile}_{GAU}$, $tRNA^{Val}_{UAC}$, $tRNA^{Ala}_{UGC}$, $tRNA^{Lys}_{UUU}$ and $tNRA^{Gly}_{UCC}$ (5,27). Our results show that the presence of long introns does not seem to interfere with the expression of these tRNA genes, since all these tRNAs are found in relative high amounts.

In bean, seven tRNA genes are present in two copies, as they are located in the inverted repeat region, namely $tRNA^{Leu}_{CAA}$, $tRNA^{Ile}_{GAU}$, $tRNA^{Ile}_{CAU}$, $tRNA^{Ala}_{UGC}$, $tRNA^{Val}_{GAC}$, $tRNA^{Arg}_{ACG}$ and $tRNA^{Asn}_{GUU}$ genes (20). Our results show that the corresponding tRNAs are not found in higher amounts than the tRNAs coded for by only one gene ; in fact $tRNA^{Leu}_{CAA}$ (coded for in the inverted repeats) occurs in lower amounts than $tRNA^{Leu}_{UAA}$ and $tRNA^{Leu}_{UAG}$ (coded for in the single copy region). Similar results on the relative concentrations of $tRNAs^{Leu}$ have been found in soybean (28).

It appears that, as already described in **E.coli** and yeast (1), there is in chloroplasts a good adjustment of the tRNA population to the codons which must be translated. Such an adjustment is probably necessary to ensure maximum efficiency in chloroplast protein biosynthesis. In some animal tissues, such as the posterior silk gland of **Bombyx mori** which synthesizes fibroin (a protein which has an unbalanced amino acid composition), there is a functional adaptation of the tRNA population to the codon frequency (29). An adjustment between tRNA population and codon usage has also been reported in maize endosperm which synthesizes zein, a protein rich in glutamine, leucine and alanine (30). But how this adjustment is achieved is not known, and further studies are required to elucidate the mechanisms controling the transcription of tRNA genes and the post-transcriptional processes in chloroplasts.

ACKNOWLEDGEMENTS

The technical assistance of Mrs A. Cosset is gratefully acknowledged. This research was partially supported by a grant to Dr. D.T.N. Pillay.

REFERENCES

1. Ikemura, T. (1985) Mol. Biol. Evol. 2, 13-34.
2. de Boer, H.A. and Kastelein, R.A. (1986) in Davis, J., Reznikoff, W.S. and Gold, L. Eds, From gene to protein ; steps dictating the maximal level of gene expression. Butterworths, Stoneham, Mass., 225-283.
3. Holm, L. (1986) Nucl. Acids Res. 14, 3075-3087.
4. Steinmetz, A. and Weil, J.H. (1986) in Marcus, A. ed, Biochemistry of plants, Academic Press, New York, in press.
5. Wakasugi, T., Ohme, M., Shinozaki, K. and Sugiura, M. (1986) Plant Mol. Biol. 7, 385-392.

6. Shinozaki, K., Ohme, M., Tanaka, M., Wakasugi, T., Hayashida, N., Matsubayashi, T., Zaita, N., Chunwongse, J., Obokata, J., Yamaguchi-Shinozaki, K., Ohto, C., Torazawa, K., Meng, B.Y., Sugita, M., Deno, H., Kamogashira, T., Yamada, K., Kusuda, J., Takaiwa, F., Kato, A., Tohdoh, N., Shimada, H. and Sugiura, M. (1986) EMBO J. 5, 2043-2049.
7. Ohyama, K., Fukuzawa, H., Kohchi, T., Shirai, H., Sano, T., Sano, S., Umesono, K., Shiki, Y., Takeuchi, M., Chang, Z., Aota, S., Inokuchi, H. and Ozeki, H. (1986) Nature 322, 572-574.
8. Burkard, G., Guillemaut, P. and Weil, J.H. (1970) Biochim. Biophys. Acta 224, 184-198.
9. Guillemaut, P., Burkard, G., Steinmetz, A. and Weil, J.H. (1973) Plant Sci. Lett. 1, 141-149.
10. Guillemaut, P., Steinmetz, A., Burkard, G. and Weil, J.H. (1975) Biochim. Biophys. Acta 378, 64-72.
11. Jeannin, G., Burkard, G. and Weil, J.H. (1976) Biochim. Biophys. Acta 442, 24-31.
12. Jeannin, G., Burkard, G. and Weil, J.H. (1978) Plant Sci. Lett. 13, 75-81.
13. Merrick, W.C. and Dure, III, L.S. (1972) J. Biol. Chem. 247, 7988-7999.
14. Herrmann, R.G., Bohnert, H.J., Kowallick, K.V. and Schmitt, J.M. (1975) Biochim. Biophys. Acta 378, 305-317.
15. Stutz, E. and Noll, H. (1967) Proc. Natl. Acad. Sci. USA, 57, 774-781.
16. Douce, R. and Joyard, J. (1982) in Edelman, M., Hallick, R.B. and Chua, N.H. eds, Methods in Chloroplast Molecular Biology, Elsevier Biomedical Press, Amsterdam, 239-256.
17. Gray, J.C. (1974) Arch. Bioch. Biophys. 163, 343-348.
18. Silberklang, M., Gillum, A.M. and Rajbhandary, U.L. (1977) Nucl. Acids Res. 4, 4091-4108.
19. Burkard, G., Steinmetz, A., Keller, M., Mubumbila, M., Crouse, E.J. and Weil, J.H. (1982) in Edelman, M., Hallick, R.B. and Chua, N.H. eds, Methods in Chloroplast Molecular Biology, Elsevier Biomedical Press, Amsterdam, 347-357.
20. Mubumbila, M., Gordon, K.H.J., Crouse, E.J., Burkard, G. and Weil, J.H. (1983) Gene 21, 257-266.
21. Kelmers, A.D. and Heatherly, D.E. (1971) Analyt. Biochem. 44, 486-495.
22. Burkard, G., Eclancher, B., and Weil, J.H. (1969) FEBS Lett. 4, 285-287.
23. Francis, M.A. and Dudock, B.S. (1982) J. Biol. Chem. 257, 11195-11198.
24. Osorio-Almeida, M.L., Guillemaut, P., Keith, G., Canaday, J. and Weil, J.H. (1980) Biochim. Biophys. Res. Commun. 92, 102-108.
25. Agris, P.F., Söll, D. and Seno, T. (1973) Biochemistry 12, 4331-4336.
26. Schön, A., Krupp, G., Gough, S.B.-L., Kannangara, C.G. and Söll, D. (1986) Nature 322, 281-284.
27. Steinmetz, A. and Weil, J.H. (1986) in Akoyunoglou, G. and Senger, H. eds, Regulation of chloroplast differentiation, Alan R. Liss, Inc., New York, 489-498.
28. Pillay, D.T.N., Singh, G.P. and Guillemaut, P. (1984) Gene 29, 351-354.
29. Garel, J.P. (1982) Trends Biochem. Sci. 7, 105-108.
30. Viotti, A., Balducci, C. and Weil, J.H. (1978) Biochim. Biophys. Acta 517, 125-132.